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THE DIAGNOSIS OF LATENT MALARIA

TROPICAL DISEASES BUREAU
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THE DIAGNOSIS OF LATENT MALARIA*

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THE object of our research has been to see if it is possible to diagnose malaria in the latent period, *i.e.*, when parasites are absent or very few in number in the peripheral blood.

Plehn,[3] Christophers and Stephens,[1] Thomson[7] and others considered that circumstantial evidence was given, in the diagnosis of this condition, by the presence or absence of urobilin in the urine or fæces, and by alterations in the number and variety of the leucocytes. We have, therefore, directed our attention chiefly to these two points, *viz.*, the urobilin and leucocytes.

But before proceeding to the results of our investigation we must first define exactly what is meant by latency. "The latent period in malarial fever may be defined as that afebrile period in a malarial infection when parasites are still active in the peripheral blood or deeper vessels, but are not present in the asexual stage in sufficient numbers to cause fever."

According to this definition there may be three distinct phases of latency present in an infection, *viz.*:—

(1) *The latent period of incubation.*—This includes all the afebrile days from the introduction of the sporozoites by the mosquito until the asexual parasites are sufficiently numerous to cause the first attack of fever. This period varies in the different forms of malaria; in malignant tertian

* Read at the Malaria Conference held at Madras in Nov. 1912.

infections the incubation period may be as short as 2 to 8 days, whilst in benign tertian and quartan it is usually about 14 to 20 days. In persons taking quinine prophylactically the infection may terminate in this stage, or if the dose of quinine be insufficient the attack may be delayed for some considerable time. The large number of cases occurring during the months of November and December in regimental and jail populations is, in our opinion, due to this fact, *viz.*, insufficient prophylaxis with prolonged incubation period

(2) *The latent period of recrudescence* is the afebrile period which elapses between consecutive attacks of fever in one and the same infection. In untreated cases this period may vary from 7 to 14 days, whereas in persons who have only undergone short courses of quinine treatment these afebrile periods are extremely irregular and may be prolonged over months. We regard recrudescence as due to a sudden increase in number of the asexual parasites already existing in the body. In the latent periods between recrudescences the asexual parasites are not present in the blood in sufficient numbers to cause fever. Every now and then a sudden increase occurs in the number of these asexual parasites, and fever results. During this latent period of recrudescence a few asexual forms are often found in the peripheral blood after careful examination by the thick film method of Ross and Thomson.(4)

Figure 1 shews these exacerbations in the asexual cycle.

(3) *The latent period of relapse.*—The term relapse should be carefully differentiated from that of recrudescence. We regard relapse as occurring after a replacement of the asexual by the sexual cycle (sporogony); and as being due to a reversion to the asexual cycle (schizogony) by a process of parthenogenesis (or self-fertilization) in the female gametes. This conclusion is based upon a study of parthenogenesis in the gametes of the analogous Halteridium parasite of the pigeon.

In an untreated case of malaria, the initial fever and the recrudescences are due to multiplication of the asexual broods (schizonts).

After 6 to 10 days from the commencement of the fever, when the patient is developing a certain amount of tolerance to the parasite, sexual forms (gametes) make their appearance in the peripheral blood. These are resistant forms, and with their entry the fever abates. The gametes are produced from the merozoites of the asexual cycle, and as the gametes themselves undergo no multiplication, the asexual cycle becomes exhausted. The peripheral blood becomes clear of asexual forms, but the gamete forms persist for some considerable time.

If, owing to some extraneous cause, as for example chill or the inoculation of vaccines, the resistance of the patient is now lowered, the female gametes give rise, by parthenogenesis, to new asexual forms, which multiply in the usual manner, and cause fever. This constitutes a "relapse," and Figure 2 shews this diagrammatically.

It therefore follows that the simultaneous presence of gametes and trophozoites in the peripheral blood of a malarial patient, who has not had fever for some considerable time previously, suggests that a relapse is about to occur.

The population we examined was an Indian male adult one, taken at random in the order of arrival at the Pasteur Institute. Only those who appeared to be in health, and not suffering from sepsis as the result of dog bite, were examined. The examinations were made on the day of arrival and within half an hour after the first inoculation.

Four hundred and sixty-four adult Indian males were examined with regard to their previous history of fever and their urines were examined for urobilin. In 187 of these a simultaneous total and differential leucocyte count was also made. The history of previous fever in our patients was very difficult to obtain with any degree of exactness. Many of them could not fix the date of the attack with any certainty, and gave correspondingly indefinite answers. At the commencement of the investigation, when a large amount of urobilin was found in the urine, the patient was re-questioned and often admitted having had fever recently. But after the first 50 cases or so, this was not repeated in order to avoid bias.

Besides the above cases, 55 cases of enlarged spleen were especially selected and further examined.

None of our cases had fever on them at the time of the examination.

Urinary Urobilin.

The method we employed was Schlesinger's test. This test is easily carried out and is very accurate, and demonstrates the slightest trace of urobilin in the urine. It is as follows :—

To the urine in a test tube, an equal quantity of a saturated solution of zinc acetate in absolute alcohol is added and the mixture well shaken. Then 4 to 10 drops of Gram's iodine solution are added and the fluid stirred. The mixture is then filtered through filter paper. The filtrate, if urobilin is present, shews a greenish fluorescence, which varies in intensity with the amount of urobilin.

With a little practice one can easily form an opinion of the amount of urobilin present, and graduate the test by the degree of fluorescence shewn.

We have divided the cases into three categories :—

(1) Fluorescence,—distinct or marked.
(2) Fluorescence,—faint or very faint.
(3) No fluorescence.

Urobilin is not found in the urine of healthy persons, but is found in various fevers, *e.g.*, pneumonia, and the late stages of typhoid and Malta fevers, when secondary anæmia has supervened, in cases of hepatic deficiency such as suppurative hepatitis, in anæmias especially in pernicious anæmia, and in cases of internal hæmorrhage.

In malaria the secretion of urobilin does not occur during the latent period of incubation, it commences with the fever and increases to its maximum output (1·5 to 2 gms. per diem according to Thomson, [6,7]) about two to three days after the attack of fever, and remains at a high level during the latent period of recrudescence. During the latent period of relapse the urobilin output is at first at a high level and then gradually falls. In the few cases we have investigated on this point the output is apt to be suddenly raised from time to time without obvious cause. We hope to be able to investigate this point further in order to see whether it gives any indication as to how long quinine treatment should be continued.

We agree with Simpson,[5] that the output is more marked in malignant tertian than in benign infections, and with De Jonge[2] that it is temporarily increased by giving quinine, and then rapidly falls when the patient is thoroughly under the influence of this drug.

Simpson regards the fæcal urobilin as the more important channel of urobilin elimination, but we have not investigated this point, owing to its practical difficulties in the type of patient dealt with.

Table 1 gives the result of our investigation on 464 individuals. Of those who gave a history of fever within the preceding three months 58 per cent. shewed a considerable amount of urobilin present in their urines. Of those who gave a history of fever from 4 to 6 months previously 42 per cent. still gave a distinct urobilin reaction. On the other hand, of the 93 persons who denied all history of fever only 13 per cent. gave a distinct urobilin reaction. This table accordingly shews that there is a distinct relationship between a history of recent fever and the amount of urobilin present.

TABLE I

Shews the relationship of the history of fever to the amount of urobilin present.

	Fever within 3 months.	Fever from 4 to 6 months.	Fever from 7—9 months.	Fever from 10—12 months.	Fever over 1 year.	No history of fever.
Urobilin distinct ...	83	28	2	14	24	12
Urobilin faint ...	34	26	6	14	28	23
No urobilin ...	27	13	4	28	40	58
	57·6%	41·7%	16·7%	25%	26%	12·9% shewed distinct urobilin.

Table 2 shews the result of the urobilin test, the degree of splenic enlargement being taken as the index of malarial infection. Out of 464 individuals who shewed no splenic enlargement 35 per cent. shewed urobilin in considerable quantities, whilst of 55 cases with palpable spleens 64 per cent. gave a positive urobilin test, and of the 10 individuals with spleens reaching to the umbilicus all gave a very marked urobilin reaction. This table accordingly shews that there is a distinct relationship between the degree of enlargement of the spleen and the amount of urobilin present.

TABLE II

Shews the relationship of splenic enlargement to amount of urobilin.

	Spleens not enlarged.	Spleens palpable.	Spleens ½ way to umbilicus.	Spleens to umbilicus.
Urobilin distinct ...	163	16	17	10
Urobilin traces ...	131	6	3	...
Urobilin *Nil* ...	170	3	...	...
	35·1%	64%	85%	100% shewed distinct urobilin.

In *Table* 3 the same number of individuals was analysed in a different way. Those without splenic enlargement were divided into those who gave a history of fever, and those who did not. Those with enlarged spleens were regarded as those certainly infected with malaria. Of persons who denied having had any fever 13 per cent. shewed urobilin present in considerable quantities. Of those who had suffered from fever within the previous 12 months 41 per cent. shewed considerable urobilin present. Of the individuals with splenic enlargement 80 per cent. gave a positive result to this test.

TABLE III

Shews the relationship of history of fever and of splenic enlargement to amount of urobilin.

	No history of fever.	History of fever.	Enlarged spleens.
Urobilin distinct	12	151	43
Urobilin traces	23	108	9
Urobilin *Nil*	58	112	3
	12 9%	40 7%	79 6% shewed distinct urobilin.

Now what does the urobilin indicate ? It is an index of the amount of blood destruction that is going on or has taken place in some part of the body. The hæmoglobin is broken up into its constituents in the liver, and the iron-free pigments are excreted into the intestine as bile pigment. This pigment leaves the body for the most part as urobilin

in the urine and fæces. As the relationship of hæmoglobin to urobilin is known fairly accurately, the amount of hæmoglobin that is destroyed can therefore be estimated. Roughly 25 gms. of hæmoglobin yield 1 gm. of urobilin.

Defective action of the liver is very marked in bilious remittent fever, and we believe that the glandular activity of the liver is frequently impaired in the other clinical forms of malaria. Hence the products of hæmoglobin destruction cannot be properly dealt with by the liver, and are excreted by the kidneys as urobilin. Urobilin will accordingly appear in the urine in considerable quantities when the blood destruction has been great, *i.e.*, immediately after an attack of fever, or when the glandular activity of the liver is impaired, as in chronic malarial cachexia.

It should be borne in mind that the presence of urobilin is not a specific test, comparable with that of finding parasites in the blood, but no more is a rise in temperature specific. As we are dealing only with the diagnosis of malaria in apyrexial periods, and as, with the exception of the anæmias, malaria is the commonest cause in the tropics of blood destruction, therefore in such periods the presence of urobilin in the urine becomes a test for latent malaria.

The Leucocytes.

Thomson, (6) from his researches on the leucocytes in malarial infection, enunciated the following laws :—

(1) During active malarial fever the number of the leucocytes in the peripheral blood is below normal and varies more or less inversely with the temperature.

(2) In malarial fever the curve representing the percentage of total mononuclear leucocytes is an exact inverse of the temperature curve.

(3) In convalescent or apparently cured malarial cases, transient periodic leucocytoses occur in the peripheral blood, and these leucocyte fluctuations arise chiefly from a polynuclear variation.

He goes on to state :—" This post-malarial leucocyte phenomenon occurred without exception in the 40 cases examined, and would seem to be an infallible sign of previous malaria, since, so far, it has not been observed in any other disease."

Our results bear this out. Figure 3, Table 4, shews the total leucocyte counts in percentages in 215 cases. One hundred and thirty

of these gave a history of fever within a year, and are shewn on the continuous curve. Eighty-five denied all history of fever, and are shewn on the dotted curve. From this one sees that with recent malaria there is a tendency for either a leucopenia or leucocytosis to be present, and that even although all temperature indications have disappeared.

Figure 4, Table 5, shews the graph of 217 cases. Of these in 149 cases urobilin was present, and in 68 urobilin was absent. The dotted curve represents in percentages those without urobilin, and here the same point comes out, that individuals shewing urobilinuria have frequently a leucopenia, or more rarely a leucocytosis present.

Figure 5, Table 6, shews the frequency distribution of cases that have or have not urobilin present, in relation to their total mononuclear count. The dotted curve represents, in percentages, the 56 individuals out of 187 who had no urobilin present. This shews that a higher mononuclear reading is obtained in cases that have urobilin than in those that have not.

The same fact is brought out on comparing the cases that gave a history of fever with those that did not. Here, again, we see that the leucocyte phenomenon is closely correlated with the history of fever, and also with the presence or absence of urobilin. See Figure 6, Table 7.

These fluctuations in the leucocytes, with a high or low mononuclear count, described by David Thomson,[6,7] are of value in helping us to diagnose latent malaria. But it must be remembered that, owing to the great fluctuation which occurs, single counts are of little value, whereas several counts taken 6 hourly for 2 or 3 days would give a truer picture.

Christophers and Stephens[1] consider that a hyaline or a large mononuclear count of 15 per cent. and over is an aid in diagnosing a recent attack of malarial fever. Figures 7 and 8 shew the result of our investigation on the above 187 cases with regard to this point. The continuous curves indicate the percentage frequency of cases that had fever (Figure 7)—or urobilin (Figure 8), whilst the dotted curves shew those cases that did not have fever (Figure 7)—or urobilin (Figure 8). These curves do not bear out the above statement of the value of a 15 per cent. large mononuclear count but shew that one may get either a high or a low count of these corpuscles in latent malaria. We believe that the increase in these mononuclear cells is not a real increase but is an apparent one, due to the fluctuations in the polymorphonuclear leucocytes.

Figure 9 shews these leucocyte variations in latent malaria. If the patient gives a history of morning rises of fever in past attacks we find that in the latent stage the morning count usually shews a leucopenia ; thus of 4,000 leucocytes there may be 2,000 polymorphonuclears, 50 per cent., and 2,000 mononuclears (large and small), 50 per cent., whereas at 4 P.M. an hour which is usually afebrile in such cases, there is a leucocytosis of 20,000, of which 13,000 or 65 per cent. may be polymorphonuclears and 3,000 or 15 per cent. mononuclears. The increase is thus chiefly in the polymorphonuclears and not in the mononuclears. Our results shew this to be the case (see Figures 3 and 4). As our examinations were usually carried out in the mornings at 11 A.M. (the commonest time for the febrile attack being from 11 A.M. to 2 P.M.), the total counts which we obtained shewed a preponderance of leucopenic cases. Had our investigations been done in the afternoons, the latent cases would probably have shewn leucocytosis. This was the case in the few afternoon examinations made on cases of latent malaria. We hope to investigate these increases in the polymorphonuclears according to Arnett's classification, in order to see how these variations occur.

In latent malaria the following examinations should be carried out in order to establish a diagnosis :—

(1) Examination as to previous history of malaria.
(2) Examination as to duration of quinine treatment.
(3) The presence or absence of urobilin in the urine.
(4) The examination of the blood by the thick film method of Ross and Thomson for presence or absence of parasites.
(5) Total leucocyte count.
(6) Differential leucocyte count.
(7) Examination of the blood at the time of day at which the attack was said to have occurred and again some hours later to see if any marked fluctuations in the leucocytes occur.

Summary.

(1) The presence of urobilin in the urine in large quantities indicates that hæmoglobin is being destroyed. The blood destruction,—except in certain well defined diseases,—is due to destruction of erythrocytes by malarial parasites. If there is no fever present, the patient is in the latent stage of the disease.

(2) In the latent period of relapse the asexual cycle is absent in the peripheral blood, and gamete formation is occurring. The urobilin consequently is diminished in amount, unless the functions of the liver are impaired, in which case urobilin may be increased.

(3) The absence of urobilin from the urine indicates either that blood destruction is not occurring, or that the liver is still able to deal with the products of hæmoglobin destruction. In such cases other tests for latent malaria must be applied.

(4) A leucopenia, of 2,000 to 7,000 per c. mm., or a leucocytosis, 16,000 or more, in the absence of other causes is suggestive of latent malaria.

(5) A high relative mononuclear percentage is also suggestive that protozoal parasites are present.

(6) A fluctuating leucocyte count, with a high mononuclear percentage at the leucopenic stage is very suggestive that malarial parasites are still present somewhere in the body.

(7) The increase in the total mononuclears and hyalines is not a real one, but apparent only, due to temporary decrease in the number of the polymorphonuclear leucocytes.

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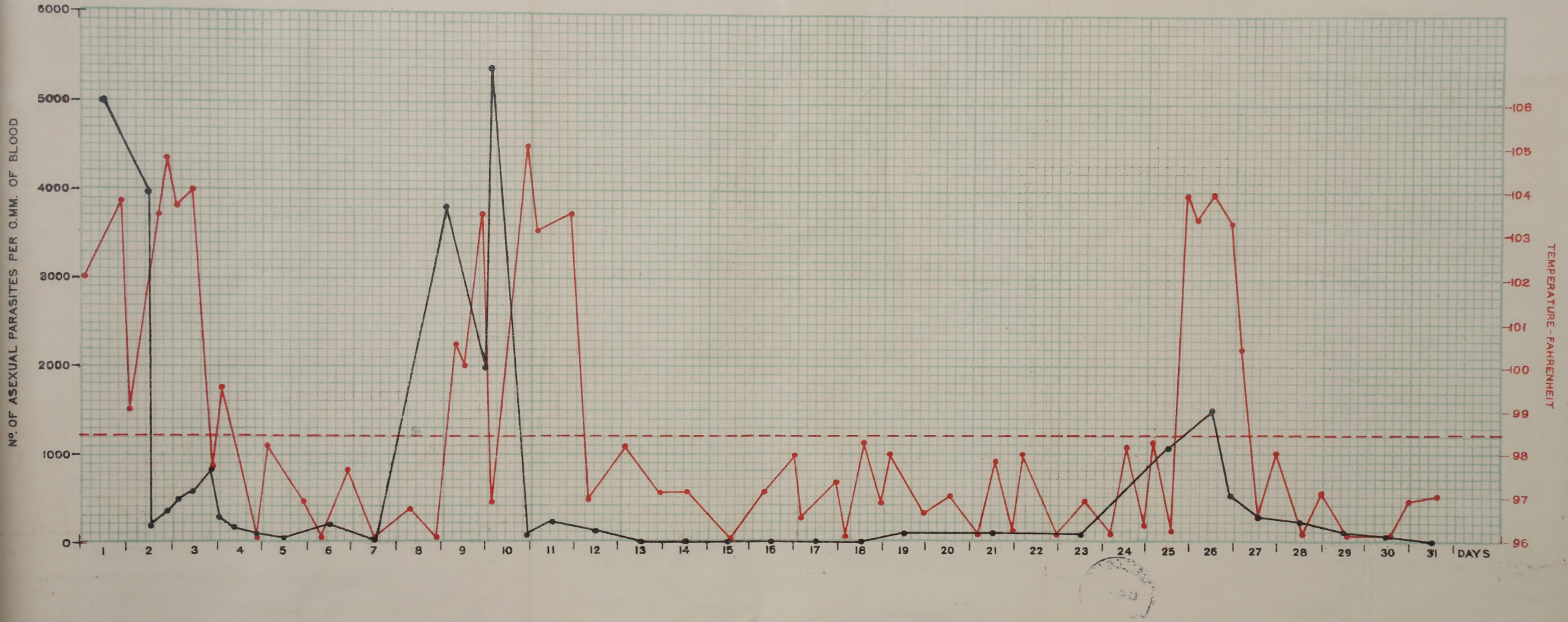
FIGURE I.

Shewing two recrudescences in a case of malaria.



No. OF ASEXUAL PARASITES PER C.MM. OF BLOOD
10000
8000
6000
4000
2000
1000
0

FIGURE II.

Shewing a relapse.

PARASITES PER C.M.M. OF BLOOD
ASEXUAL PARASITES
TEMPERATURE IN DEGREES FAHRENHEIT

FIGURE III.

Percentage distribution curve of the number of leucocytes in cases.—
(*a*) that gave a history of fever (continuous graph);
(*b*) that gave no history of fever (dotted graph).

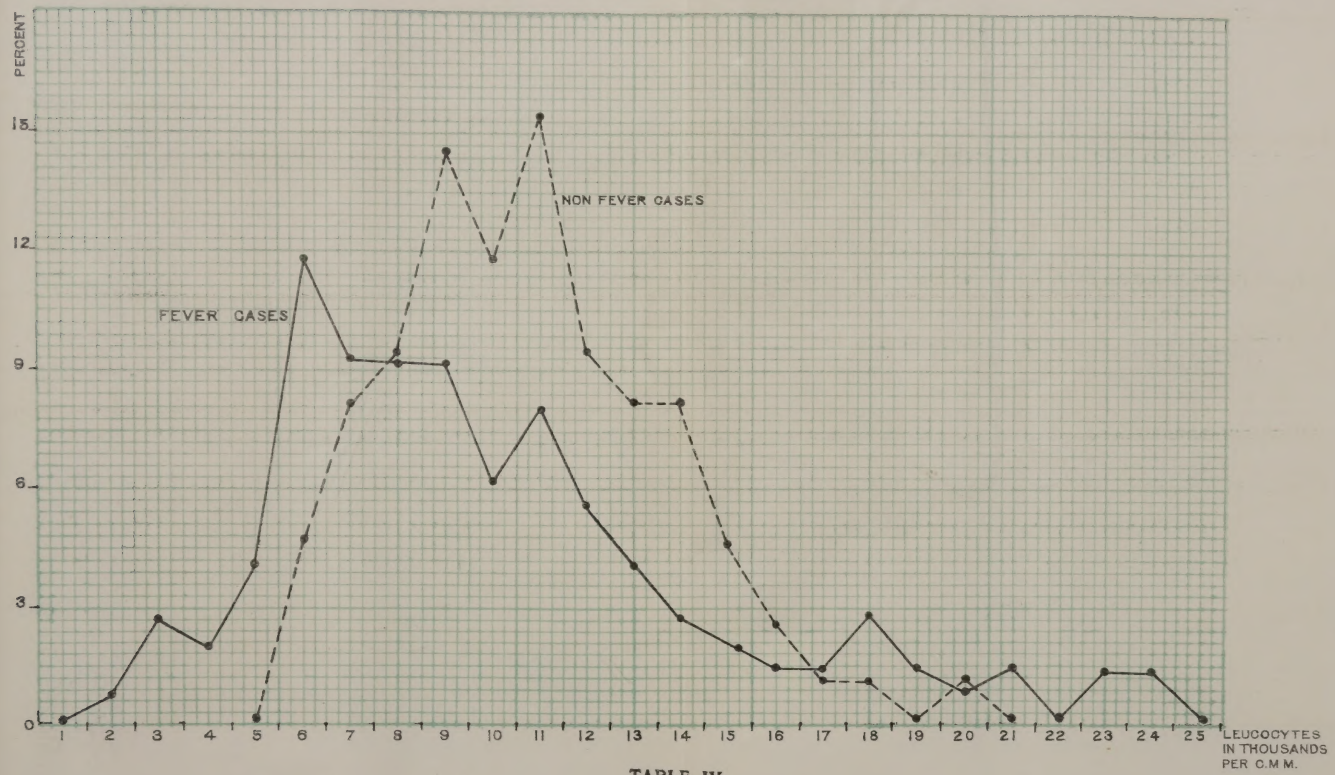


TABLE IV.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Leucocytes in 1,000's per c.mm.
History of fever within 1 year	0	1	4	3	6	7	14	14	14	9	12	8	6	4	3	2	2	4	2	1	2	0	1	1	0	Total fever cases 130.
No history of fever	0	0	0	0	0	4	7	8	12	10	13	8	7	7	4	2	1	1	0	1	0	0	0	0	0	Total no history of fever cases 85.

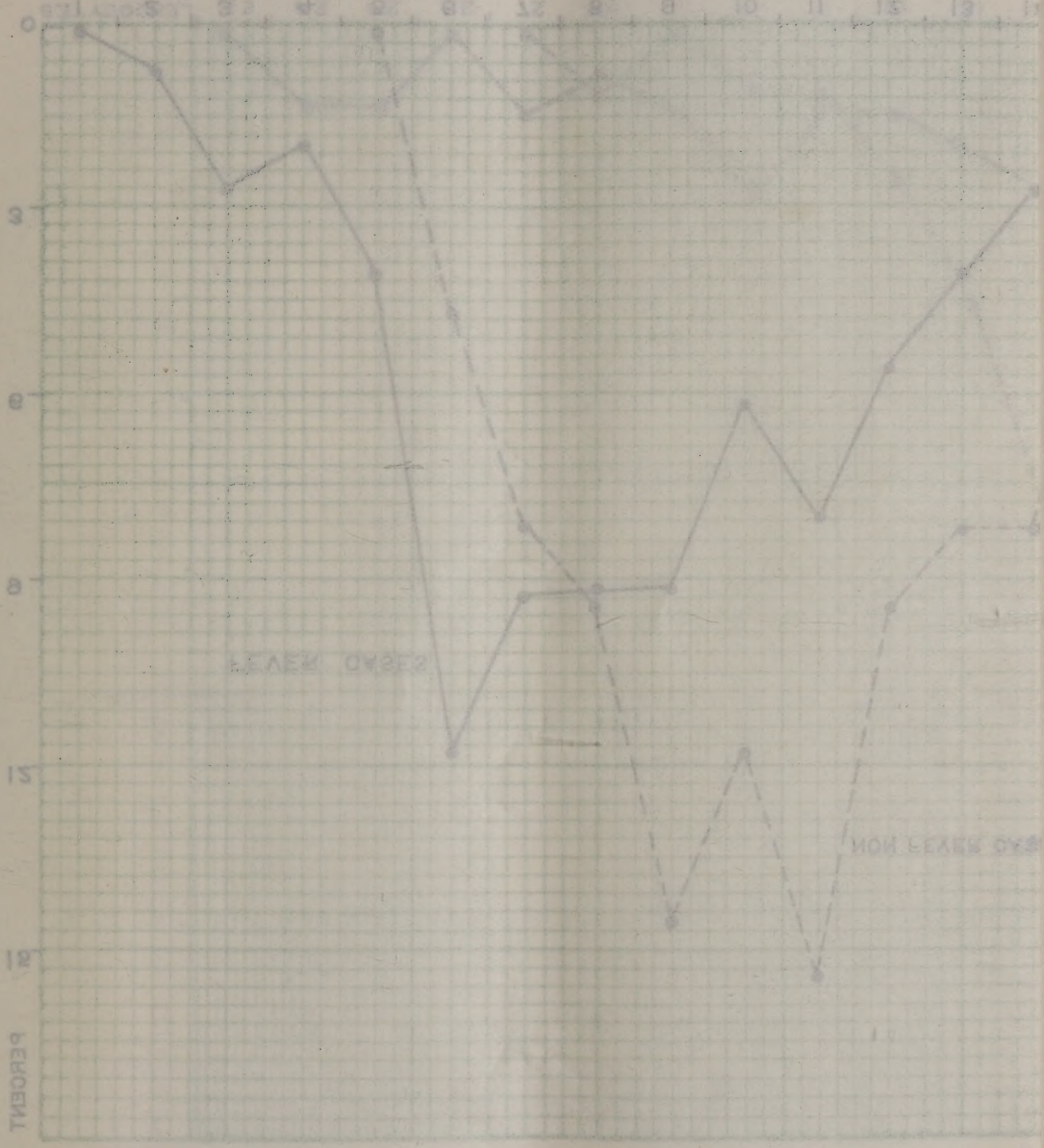

0
4
8
12
16
TENDERS

FIGURE IV.

Percentage distribution curve of the number of leucocytes in cases—
(*a*) shewing urobilin (continuous graph);
(*b*) not shewing urobilin (dotted graph).

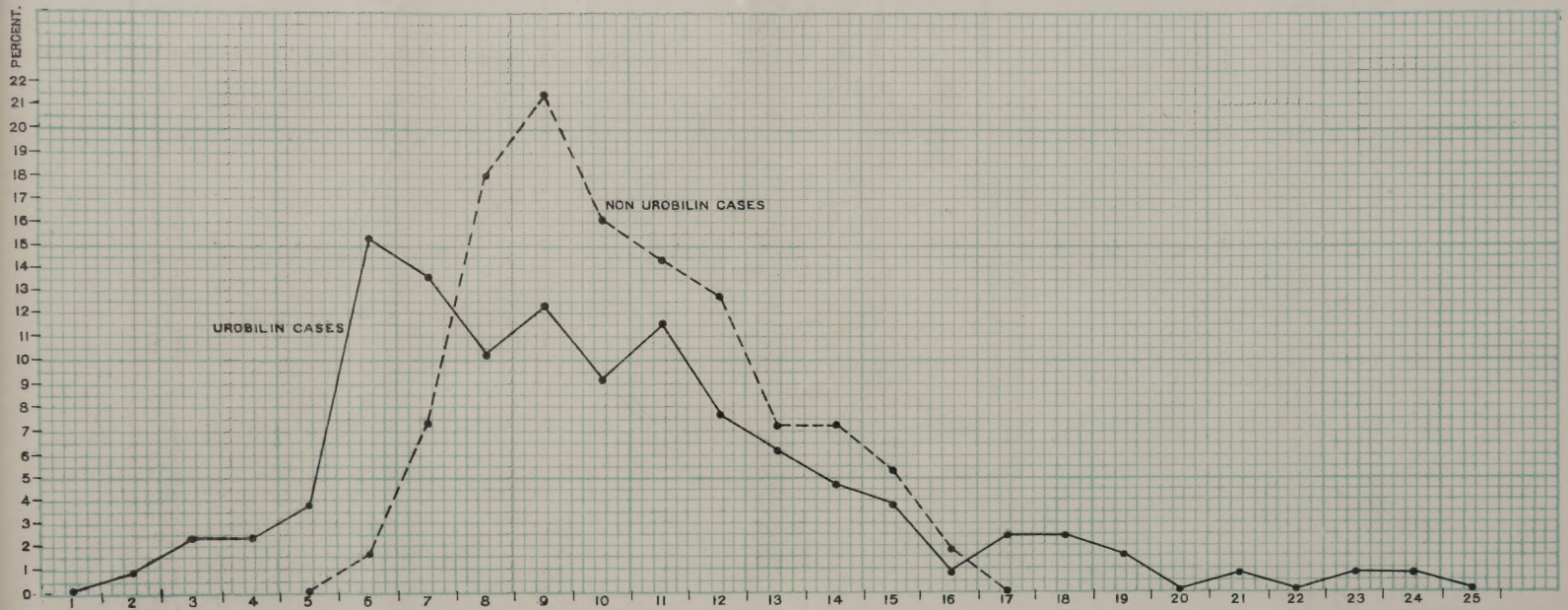


TABLE V.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Leucocytes in 1,000's per c.mm.
Urobilin present ...	0	1	3	3	5	20	18	13	16	12	15	10	8	6	5	1	3	3	2	0	1	0	1	1	0	Total urobilin cases 139.
Urobilin absent ...	0	0	0	0	0	1	4	10	12	9	8	7	5	4	4	3	1	0	0	0	0	0	0	0	0	Total non-urobilin cases 68

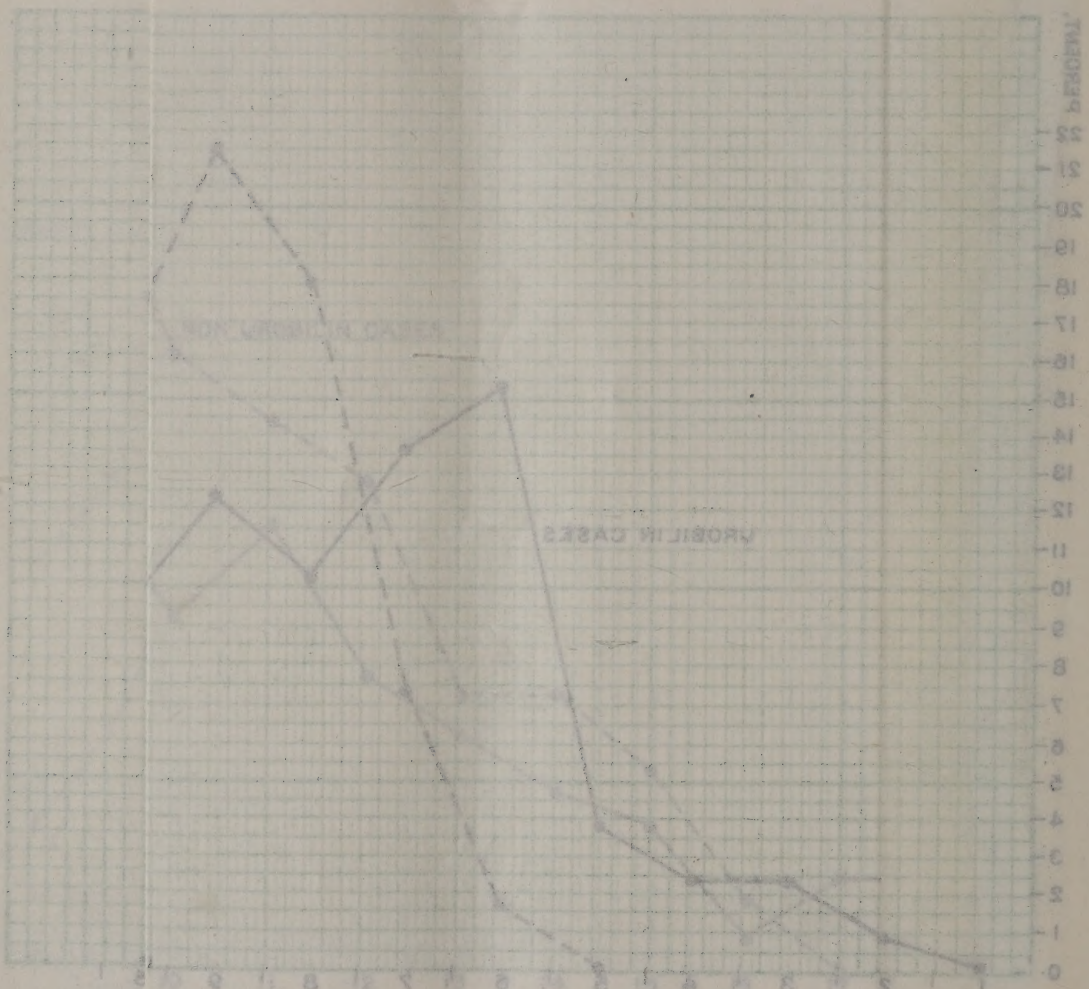

UROBILIN CASES
NON UROBILIN CASES

FIGURE V.

Shewing total mononuclear percentage in—
(*a*) Urobilin + cases (continuous graph);
(*b*) Urobilin − cases (dotted graph).

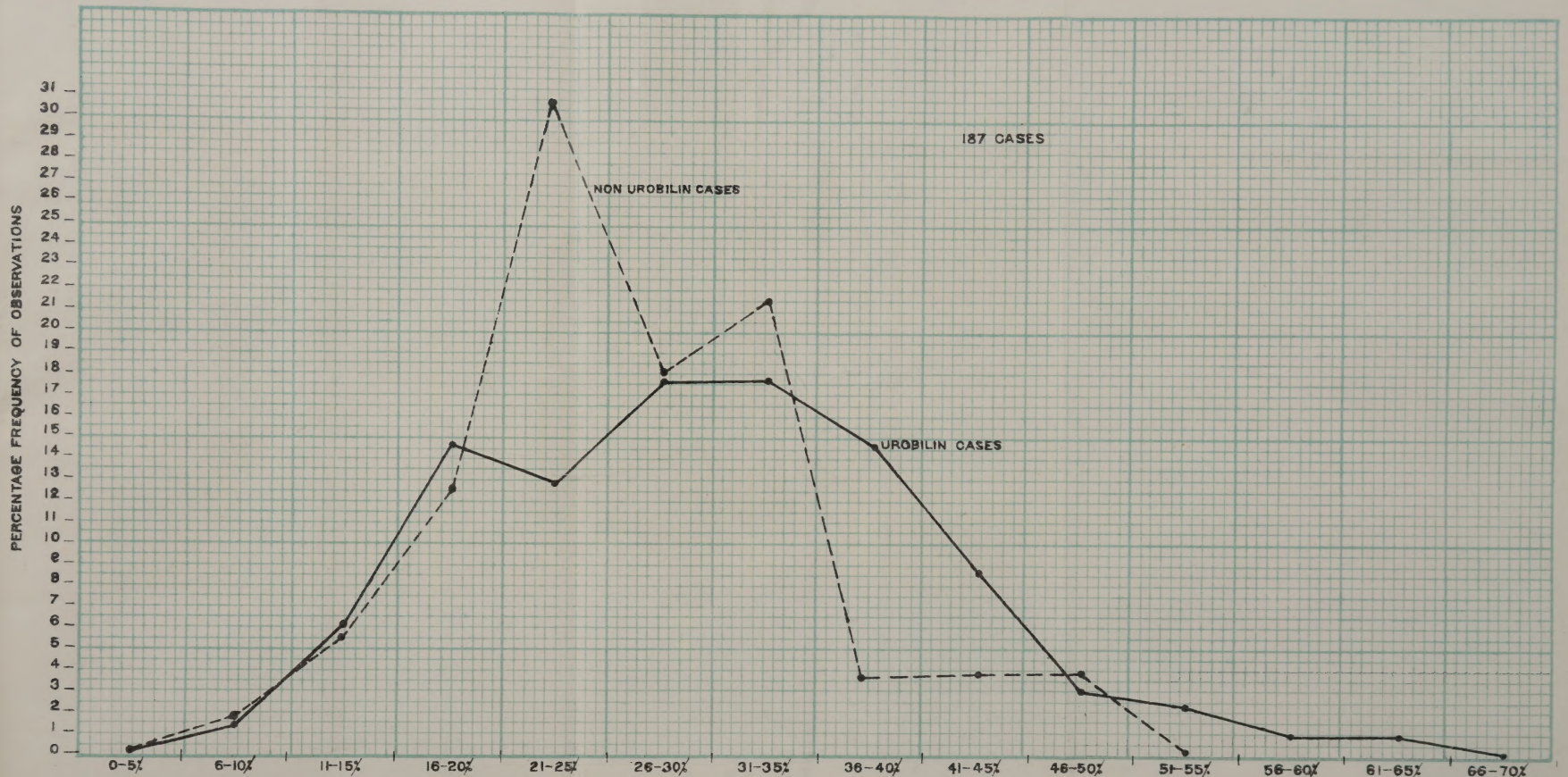


TABLE VI.

		% 0-5	% 6-10	% 11-15	% 16-20	% 21-25	% 26-30	% 31-35	% 36-40	% 41-45	% 46-50	% 51-55	% 56-60	% 61-65	% 66-70	
Mononuclear percentage count.	Urobilin + ...	0	2	8	19	17	23	23	19	11	4	3	1	1	0	131 cases.
	Urobilin − ...	0	1	3	7	17	10	12	2	2	2	0	0	0	0	56 cases.

NUMBER OF OBSERVATIONS
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
0-5
6-10
11-15
16-20
21-25

FIGURE VI.

Shewing total mononuclear percentage in cases—
(*a*) with history of fever;
(*b*) with no history of fever.

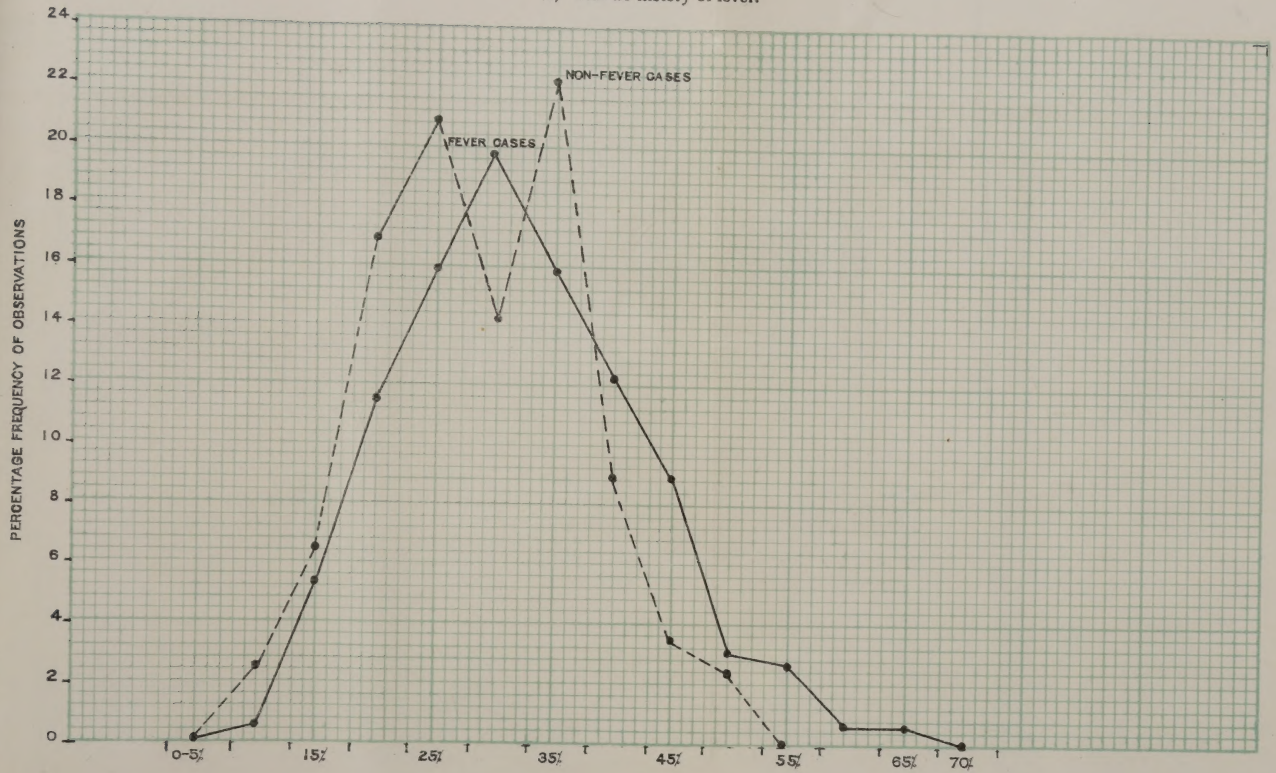


TABLE VII.

	% 0-5	% 6-10	% 11-15	% 16-20	% 21-25	% 26-30	% 31-35	% 36-40	% 41-45	% 46-50	% 51-55	% 56-60	% 61-65	% 66-70	
Within 1 year	Fever +	0	1	6	13	18	22	18	14	10	4	3	1	1	0	111 cases.
	Fever −	0	2	5	13	16	11	17	7	3	2	0	0	0	0	76 cases.

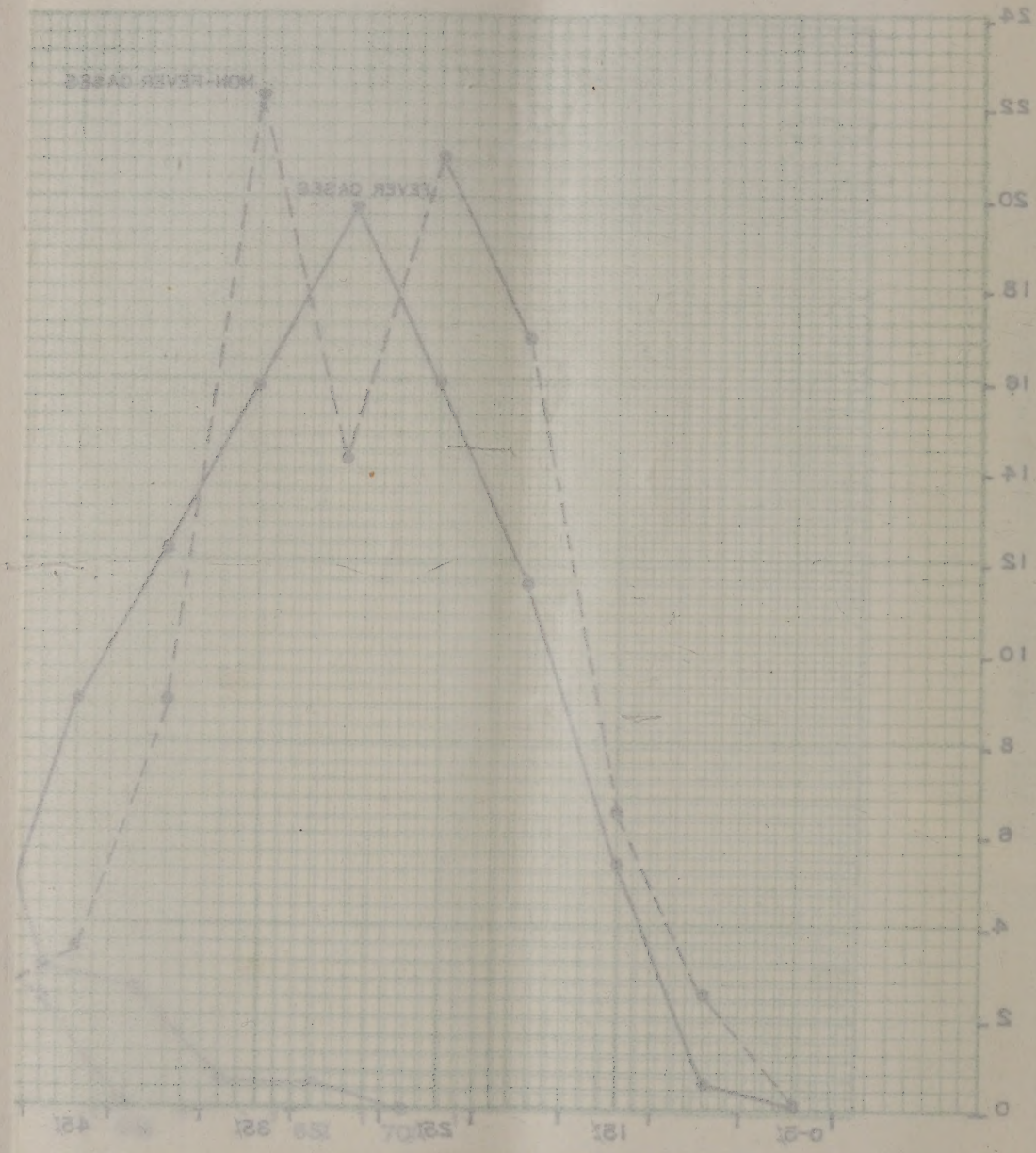
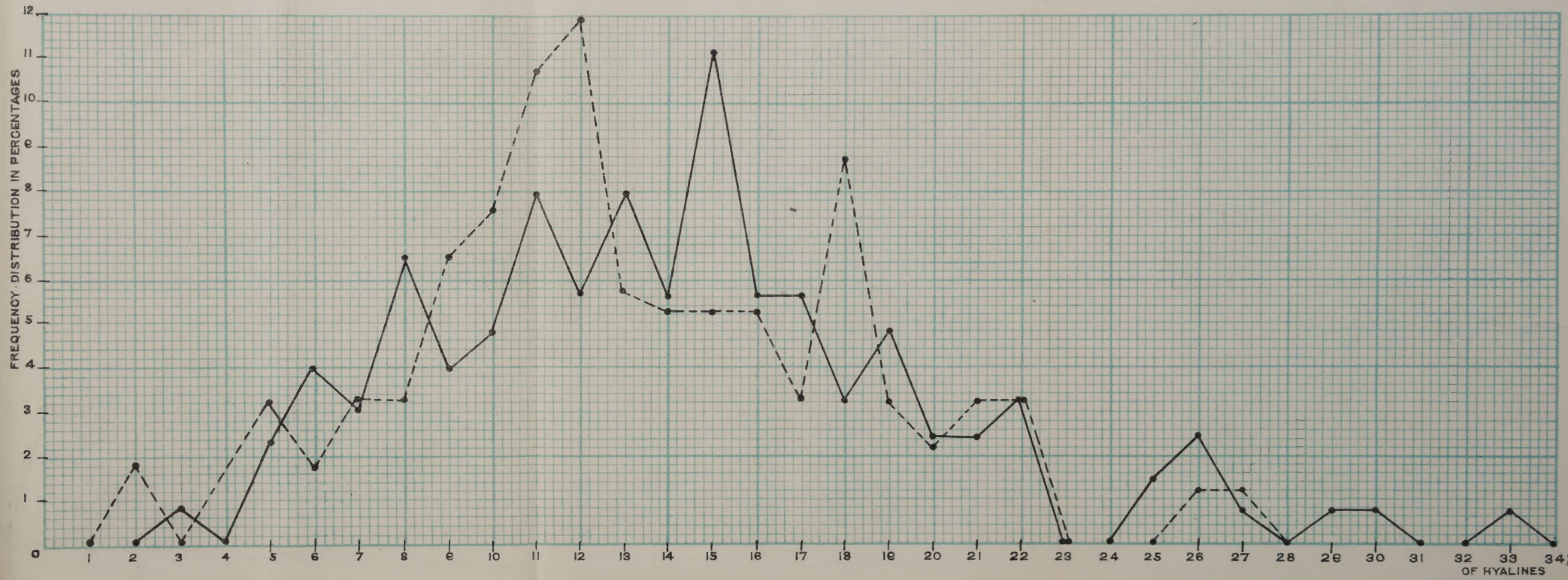

NON-FEVER CASES
FEVER CASES
PERCENTAGE FREQUENCY OF OBSERVATIONS
0
2
4
6
8
10
12
14
16
18
20
22
24

FIGURE VII.

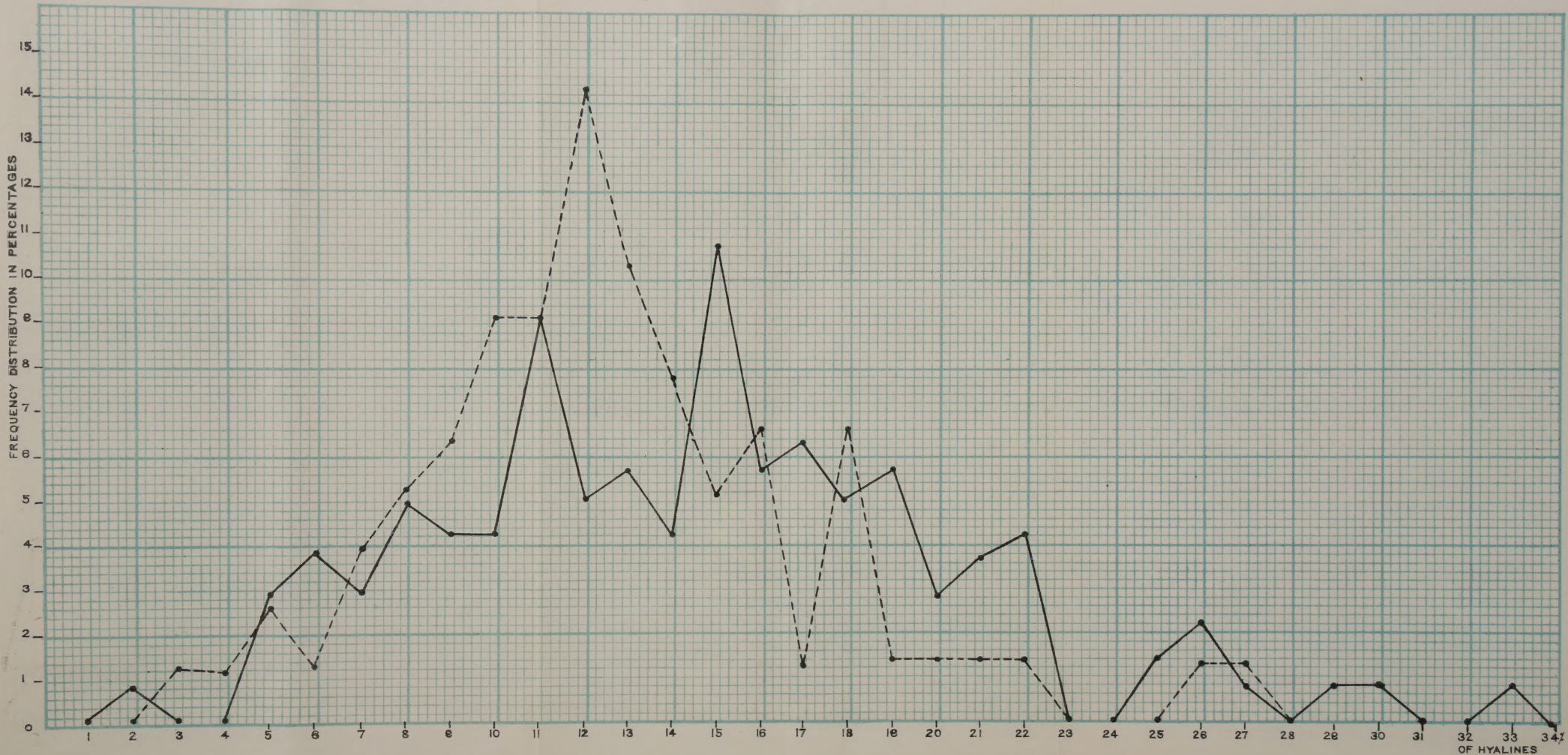
Cases with history of fever (continuous graph.)
Cases with no history of fever (dotted graph.)



FREQUENCY DISTRIBUTION IN PERCENTAGES

Figure VIII.

Urobilin cases (continuous graph)
Non-urobilin cases (dotted graph)



STUDENTS DISTRIBUTION IN PERCENTAGES

FIGURE IX.

Shewing the fluctuations in the leucocytes in latent malaria
The chart represents the morning and afternoon leucocyte count
of a case of malaria cacheria on fourteen consecutive days.